The first total synthesis of moracin O and moracin P, and establishment of the absolute configuration of moracin O†

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The first total synthesis of the naturally occurring benzofurans, moracins O and P was achieved using a Sonogashira cross coupling reaction followed by *in situ* cyclization, and the absolute configuration of natural moracin O was established.

Mori Cortex Radicis, the root bark of some *Morus* species, has been used in oriental medicine as an antidiabetic, a diuretic, an expectorant and a laxative agent.[1] Various prenylated flavonoids, benzofurans and other phenolic compounds[2] with biological activities, including cytotoxicity,[3] COX-1 and 2 inhibition,[4] and NO production,[5] have been isolated from these species.

In search of small molecule inhibitors against hypoxia-inducible factor (HIF)-1,[6] which is a master regulator of the adaptation process of cancer cells to tumor hypoxia, a bioassay-guided fractionation study with a hypoxia response element (HRE) reporter assay on natural products has been conducted. Some moracins were found to exhibit potent inhibitory effects in cell-based HRE assays in human hepatocarcinoma Hep3B cell lines. (−)-Moracin O (**1**; Fig. 1) exhibited the strongest inhibitory activity (IC_{50} = 0.14 nM), while (−)-moracin P (**2**) had an IC_{50} value of 0.65 nM.[7]

Moracins O and P were first isolated in 1998 from an acetone extract of cortex and phloem tissues of *Morus alba* shoots infected with *Fusarium solani* f. sp. *mori*, and their spectra were reported.[8] Interesting biological properties with a novel biosynthetic pathway made these phytochemicals an attractive synthetic target. Syntheses using simple strategies for moracin M[9] and an efficient route to moracin C have been reported.[10] To the best of our knowledge, there are no reports concerning the synthesis of moracins O or P. Emerging interest in the field of HIF inhibitors, together with the considerable biological activity of moracins O and P, have provided the motivation to complete an adaptable and scalable total synthesis of these compounds. However, the absolute configuration of moracin O has still not been confirmed.

In this communication, we first report efficient synthesis of (±)-moracins O and P *via ortho*-prenylated phenolic intermediate **6** as a common precursor. The asymmetric synthesis of (*R*)- and (*S*)-moracin O will be introduced, and the absolute configuration of bioactive natural moracin O is also demonstrated.

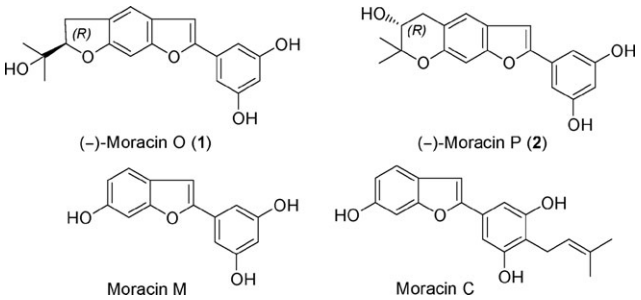
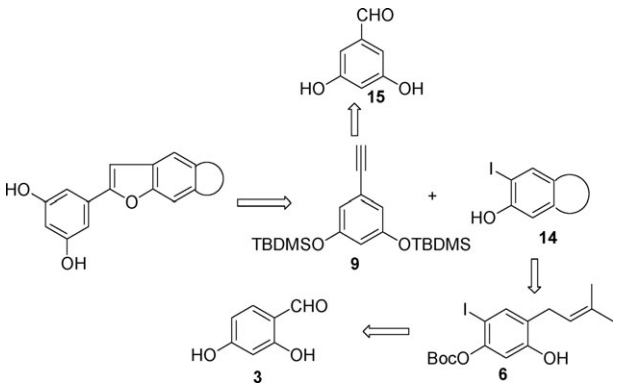


Fig. 1 Natural moracins.



Scheme 1 Retrosynthetic approach.

A retrosynthetic analysis for (±)-moracins O and P through the disconnection of the benzo[*b*]furan nucleus of these two compounds can be constructed by Sonogashira cross coupling with acetylene **9** and **14** and subsequent *in situ* cyclization (Scheme 1). The substituted acetylene, 1,3-bis-(*tert*-butyldimethylsilanyloxy)-5-ethynylbenzene (**9**) can be derived from commercially available 3,5-dihydroxybenzaldehyde (**15**).[11] It was also envisioned that nucleus **14** (a benzohydropyran in the case of moracin P; a benzohydrofuran for moracin O) could be synthesized from the common *ortho*-prenylated phenolic intermediate, **6**, which in turn can be derived from 2,4-dihydroxybenzaldehyde (**3**).

Our synthesis started with the iodination of **3** in the presence of iodine monochloride to produce 2,4-dihydroxy-5-iodobenzaldehyde (**4**; Scheme 2). In this reaction, however, we

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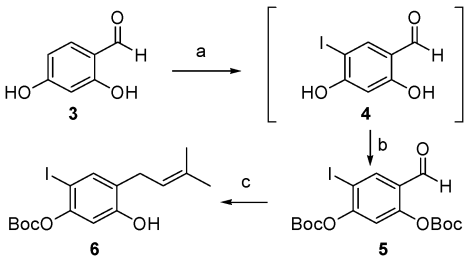
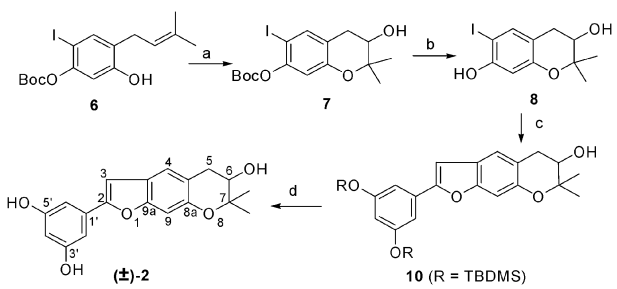


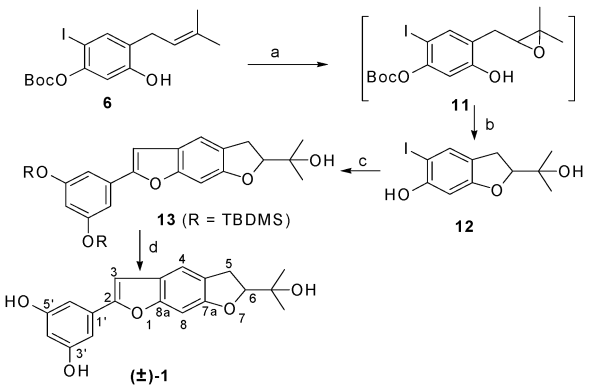
Scheme 2 *Reagents and conditions:* (a) ICl, AcOH, rt; (b) Boc_2O, K_2CO_3, 49% over steps; (c) (i) $NaBH_4$, THF, H_2O; (ii) (2-methylprop-1-enyl)-magnesium bromide, THF, −78 °C to rt.

obtained the undesired regioisomer 2,4-dihydroxy-3-iodobenzaldehyde as a side product in an inseparable 1 : 2 ratio. This mixture was confirmed by the ^{1}H NMR spectrum, leading us to believe that we in fact had two regioisomers. This crude mixture was protected with a Boc group using Boc_2O and K_2CO_3. At this stage the two isomers could be easily separated by column chromatography and the doubly protected iodobenzaldehyde, **5**, was obtained in an overall yield of 49% over two steps from **3**. The reduction of **5** with $NaBH_4$ in THF–H_2O (19 : 1) afforded an intermediate benzyl alcohol, which, after work up, but with no additional purification, was allowed to react with 2-methylpropenyl magnesium bromide to provide the prenylated derivative, **6**[12] in a 38% yield over two steps. This compound served as a common intermediate for the synthesis of both moracin O and moracin P.

For the synthesis of (±)-moracin P, *in situ* epoxidation with *m*-CPBA, followed by [6-*endo*-trig]-closure under acidic conditions (*p*-TSA), furnished Boc-protected benzohydropyran **7** in a 70% yield (Scheme 3). During initial trials of the Boc deprotection of **7** under HCl–dioxane conditions, we observed simultaneous de-iodination,[13] but under mild conditions, *i.e.* with $ZnBr_2$,[14] **8** was readily obtained in an 80% yield. The reaction of **8** with alkyne **9** under Sonogashira cross coupling conditions, followed by *in situ* cyclization in dioxane, afforded benzo[*b*]furan intermediate **10** in a 36% yield. Early attempts to remove the TBDMS groups of compound **10** with TBAF, stirred overnight, yielded mixtures of products, possibly due to the strong basic conditions and/or the long reaction time, which either permitted group migration[15] or opening of the pyran ring. The same deprotection reaction with HF–pyridine complex afforded clean removal of the TBDMS groups and provided the desired racemic moracin P (**2**) in a 75% yield.



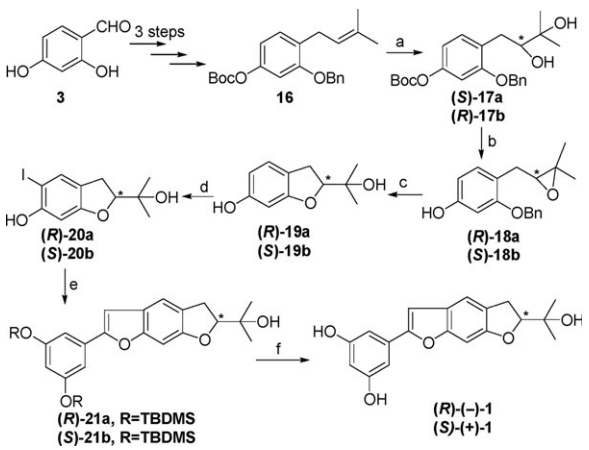
Scheme 3 *Reagents and conditions:* (a) *m*-CPBA, *p*-TSA, rt, 70%; (b) $ZnBr_2$, DCM, rt, 80%; (c) **9**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, dioxane, 85 °C, 36.9%; (d) HF–Py, THF–Py 0 °C to rt, 75%.



Scheme 4 *Reagents and conditions:* (a) *m*-CPBA, EtOAc, 0 °C; (b) LiOH, MeOH, 56.7% over 2 steps; (c) **9**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, dioxane, 85 °C, 31%; (d) HF–Py, THF–Py, 0 °C to rt, 74.8%.

After completion of the synthesis of (±)-moracin P, we turned our attention to (±)-moracin O (Scheme 4). The key step was the construction of benzohydrofuran nucleus **12**. This compound can be distinguished from its isomer, **8**, at the Boc-deprotected stage by thin layer chromatography (TLC) and was characterized by ^{1}H NMR spectroscopy as the cyclic CH_2 of compound **8** undergoes geminal coupling, while the CH_2 of compound **12** lacks this coupling. *In situ* epoxidation of **6**, followed by [5-*exo*-tet]-cyclization using $NaHCO_3$ in $CHCl_3$, was unsatisfactory for Boc-protected **12**, because it gave a product that was found to be significantly contaminated with pyran **7**. Accordingly, we addressed the reaction of intermediate epoxide **11** with LiOH, which gave **12** as the sole product in good yield. The reaction of **12** with **9**, employing Sonogashira cross coupling under basic conditions and *in situ* cyclization, afforded **13**, which upon final deprotection with HF–pyridine, provided (±)-moracin O (**1**) in a 75% yield. The NMR spectra of synthetic (±)-**1** and (±)-**2** were identical to the spectra of the corresponding natural products.[8b]

Furthermore, in order to determine the absolute configuration of natural moracin O, the asymmetric syntheses of (*R*)- and



Scheme 5 *Reagents and conditions:* (a) AD-mix-α, methanesulfonamide, *t*-BuOH–H_2O for **17a**; AD-mix-β, methanesulfonamide, *t*-BuOH–H_2O for **17b**; (b) (i) tosyl chloride, pyridine, (ii) K_2CO_3, methanol; (c) Pd black, 1,2-cyclohexadiene, EtOH; (d) ICl, AcOH; (e) **9**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, dioxane, 85 °C; (f) HF–Py, THF–Py, 0 °C to rt.

Table 1 *In vitro* inhibition of HIF transcriptional activity

Compound	IC_{50}[a]	Cell viability
(±)-**1**	6.76 nM	>30 μM
(±)-**2**	10.7 nM	>30 μM
(*R*)-(−)-**1**	0.19 nM	>30 μM
(*S*)-(+)-**1**	6.22 μM	>30 μM
(−)-**1**[7]	0.14 nM	>30 μM
(−)-**2**[7]	0.65 μM	>30 μM

[a] Values were obtained from a cell-based HRE luciferase assay (see the ESI).

(*S*)-moracin O were conducted as shown in Scheme 5. Through three steps,[16] **3** provided benzyl-protected prenylated derivative **16**. The Sharpless asymmetric dihydroxylation of **16** using commercially available catalyst AD-mix-α or β[17,18] yielded diols (*S*)-**17a** or (*R*)-**17b** in 90% and 95% ee, respectively (determined by chiral HPLC).[19] The selective tosylation of the diols (*S*)-**17a** or (*R*)-**17b** using tosyl chloride, followed by basic treatment of the resulting tosylated compound, led to exclusive formation of the desired epoxides, (*R*)-**18a** or (*S*)-**18b**, in a 45% overall yield. Debenzylation of the requisite enantiomers (*R*)-**18a** or (*S*)-**18b** using palladium black and 1,4-cyclohexadiene as a hydrogen donor,[20] provided the corresponding benzofuran compounds, (*R*)-**19a** or (*S*)-**19b**. Introduction of the iodo group at the *ortho* position of benzofuran derivative (*R*)-**19a** or (*S*)-**19b** by using iodine monochloride furnished optically active benzofurans (*R*)-**20a** or (*S*)-**20b** in good yields. This key intermediate reacted with protected ethynyl benzene compound **9** through a Sonogashira reaction by using palladium catalyst to afford chiral moracin O precursors (*R*)-**21a** or (*S*)-**21b** in moderate yields. Finally, deprotection with hydrogen fluoride in pyridine produced target compounds (*R*)- and (*S*)-moracin O in good yields. The spectroscopic data of (*R*)-moracin O were in accordance with that reported for the natural moracin O.[19b] The specific rotation of (*R*)-moracin O ($[\alpha]^{28}_{D} = -4.45$ (*c* 0.05, MeOH)) was matched to that of natural moracin O ($[\alpha]^{25}_{D} = -4.02$ (*c* 0.04, MeOH)), thereby validating the full configurational assignment of natural moracin O.

The synthetically generated racemic compounds (±)-**1** and (±)-**2**, as well as (*R*)-(−)-**1** and (*S*)-(+)-**1**, were evaluated for their effects on hypoxia-induced HIF activation by a cell-based HRE-reporter assay in Hep3B cell lines. (±)-**1**, (±)-**2** and (*R*)-(−)-**1** exhibited potent inhibition, with IC_{50} values of 6.76, 10.7 and 0.19 nM, respectively, without cytotoxicity (Table 1).

In summary, we have developed a simple and efficient protocol for the first total syntheses of (±)-moracin O (**1**) and (±)-moracin P (**2**) in overall yields of 2.21% and 2.27%, respectively. In addition, the enantioselective syntheses of (*R*)- and (*S*)-moracin O have been achieved from 2,4-dihydroxybenzaldehyde. The absolute stereochemistry was introduced by employing Sharpless' AD reaction.

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